A BACTERIAL DISEASE OF OLEANDER.

BACILLUS OLEAE (Arcang.) Trev.

CLAYTON O. SMITH.

(WITH FOUR FIGURES)

DURING the autumn of 1905 some diseased oleanders were sent from a nursery to the plant pathological laboratory of the University of California. This disease has been occasionally reported as occur-

FIG. 1.—Oleander, showing knots on stem and leaf from natural infection.

ring on young oleanders in this state. The trouble affects the stem and leaves (*figs. 1, 2*), forming large, hard, woody knots. These knots were examined by Professor R. E. SMITH and found to contain

numerous bacteria which were produced in small colonies in the tissue. The general nature of the knot and the close botanical relationship of the oleander and olive immediately suggested to him that the trouble might be caused by the same organism that produces the so-called knot or tuberculosis of the olive. The subject was assigned to the writer for further investigation. The work for the most part was done at the bacteriological laboratory of the University, and thanks are due to C. M. HARING and Professor A. R. WARD, of that laboratory, for their courtesy and suggestions during the investigation.

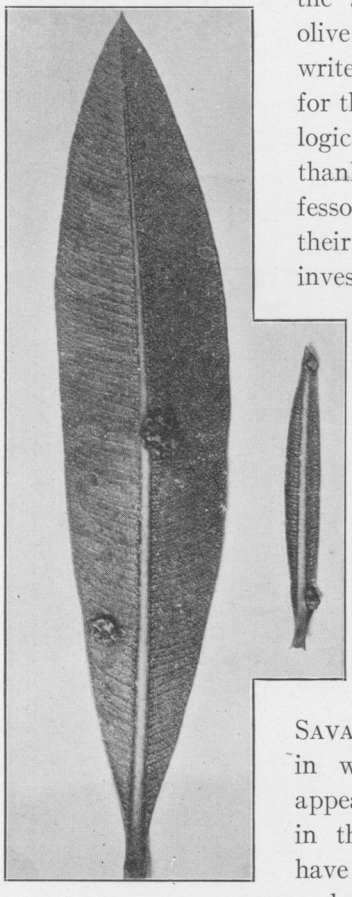

FIG. 2.—Leaves of oleander, showing knots from natural infection.

The olive knot is a disease of the branches and leaves of the olive tree. It occurs in Egypt, in the olive-growing sections of Europe bordering on the Mediterranean, and is also found in California where the olive is grown. The disease has been known for many years and is even described by Roman writers; but its bacterial origin has only been recognized since 1886, when the organism was discovered by ARCANGELI (1) and SAVASTANO (2). It is found in the knots in what may be called colonies. These appear as clearer or more transparent spots in the callus-like tissue. These growths have their origin near the cambium layer and at length become darker in color. About this colony hypertrophy of the tissue takes place, as a natural effort of the plant to heal the injury caused by the bacteria. This same process of healing would take place in mechanical injuries. The result is that much soft, spongy tissue is formed that makes a rather favorable place for new bacterial growth, which means a new

formation of callus tissue and hence an increase in the size of the knot.

These large knot-like growths of the olive may be found on the leaves, branches, and trunks, few in number or in great abundance. They must not be confused with those caused by insects, and they are also distinct from the enlargements formed on the roots of the Leguminosae by bacteria. Badly diseased trees show scant foliage, limited growth, and occasional dead branches.

The cultural characteristics of the olive knot organism have been studied by SAVASTANO (**5**) and seem to agree quite well with those observed during this study. The following is a portion of the translation of SAVASTANO'S account of the disease as published by PIERCE (**7**):

> This microorganism is a bacillus of medium size; length three to four times its width; it is isolated, but is sometimes joined into chains; the extremities are slightly rounded off. In drops of bouillon it has a distinct movement. The colony has a variable form, from round to oval, with a well-defined margin. In the beginning it is uniformly pointed; later it forms one or two peripheral circles. It is whitish by reflected light, cedar color by transmitted light. The bacillus lives well in ordinary culture media (bouillon, potato, gelatin, agar). The culture has a relatively long life; cultures made in March were still living in June. In short, degeneration begins in about three months. On potato it lives very well and develops rapidly; the colonies are at first like so many round dots, translucent straw color, which, as they develop, form on the surface of the potato a uniform stratum, translucent, and of a deeper color. The bacillus acquires greater dimensions. On gelatin plates it lives very well, with characters and forms as above indicated. In the tubes of gelatin (*a becco*) the culture presents the appearance of a uniform stratum, whitish, the margin finely bilobed, reminding one of the margin of a leaf, the whole culture taking the form of a spatulate leaf. It is slightly dichroic. In tubes of agar (*a becco*) the culture is identical with the preceding, the margin is less bilobed. The culture by needle in gelatin presents a uniform, transparent, finely pointed appearance. On the surface of the meniscus the form is irregularly rounded, with a finely lobed margin, as in the preceding.

In the study of the present disease, the organism was first isolated from the oleander in the manner described below. Inoculations were then made on the oleander and olive. Positive results were obtained in all cases. No effort was made to try inoculations on other plants. SAVASTANO (**5**), however, failed to make successful inoculation with the olive knot organisms on peach, plum, apricot,

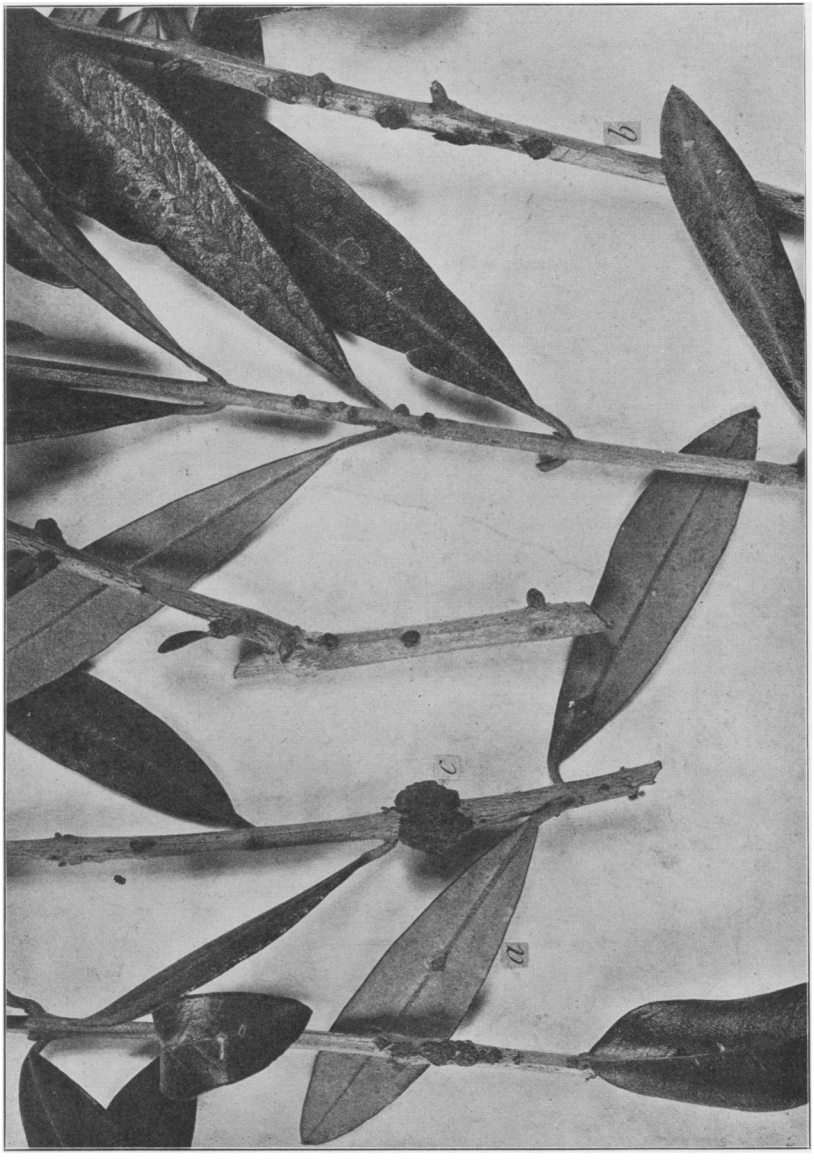

FIG. 3.—Knots on olive caused by artificial infection from pure culture: *a*, on leaves; *b*, on tree inoculated in the open; *c*, large knot and smaller knots on plants growing under glass.

grape, fig, pear, apple, bitter orange, lemon, rose, *Abies excelsa*, *A. pectinata*, and *Cedrus Libani*. He did not experiment with the oleander, however, or any other plant related to the olive.

It required considerable time in the writer's experiments for the disease to develop, though in a month's time the first indication of tissue enlargement could be observed. This continued to increase until on the olive quite a knot was formed in two month's time (*fig. 3*). Often the infection did not produce such a large knot as indicated in *fig. 3*, but smaller swellings of the tissue. In this inoculation work agar cultures, 48 hours old, were used, except on one occasion when a bouillon culture was tried. Either gave equally satisfactory results. The visible effects of the inoculations showed sooner on the oleander, but their size and the rapidity of knot-formation seems to depend upon the rapidity with which the plant is growing, as has been before observed by SAVASTANO (**4**) in his study of the olive knot. The organism grows on both the stem and the leaves. In one experiment a leaf of an oleander near the top of the plant was inoculated on the midvein; this inoculation grew well, and from it secondary natural infections resulted on the stem. It was not difficult to trace the new infections on the stem to the very base of the petiole of the diseased leaf. Infection (*fig. 4*) took place probably through the stomata and lenticels. Checks were used by making punctures with a sterile needle, but these gave no knot formations.

The lesions and growth on the hosts were quite different. On the oleander at first there was a slight enlargement of the tissue that became somewhat rounded at the point of infection. After a time, as the new growth continued, there was a splitting of the epidermis in a longitudinal direction, forming a cleft (*fig. 4*). After this a spongy growth formed, which is rather dark in color and contains numerous small colonies of the bacteria. On the olive there was the same enlargement of the tissue as in the oleander, but the formation of the new growth was much more rapid, regular knots being soon formed by the growing out of the new tissue. This took place in the olive rapidly, while in the oleander it was only in the advanced stages that this new callus tissue grew into a knot-like formation. The knots on the olive agreed in appearance with specimens of the typical knots as it occurs in California, and with the illustration as given by PIERCE (**7**), BIOLETTI (**8**), and ERWIN SMITH (**9**).

The original cultures were made from the diseased tissue of the oleander by first cutting away the outside tissue with a scapel, steri-

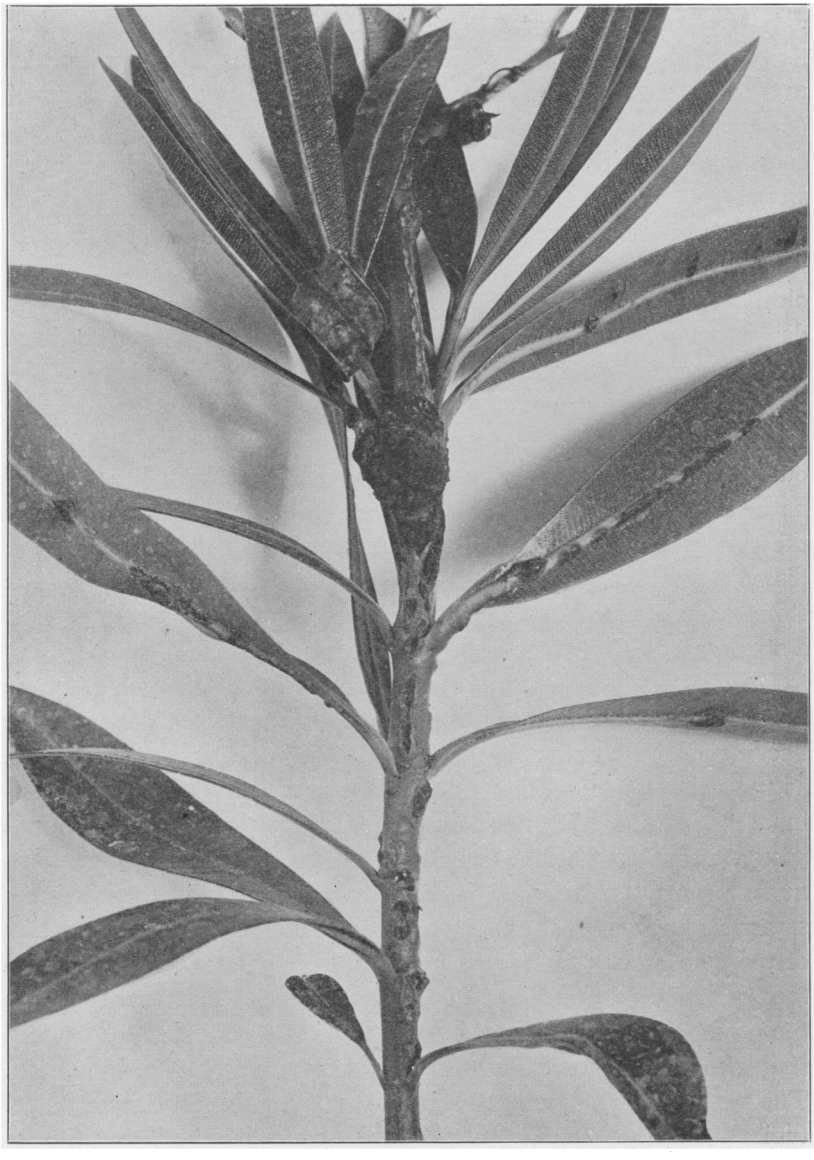

FIG. 4.—Knots on oleander leaves and stem caused by artificial infection from pure culture.

lized by flaming. When this was done, the small colonies could be seen in the tissue as dark clear places; these were touched with a sterile platinum needle, and a tube of ordinary meat bouillon was inoculated. Several such tubes were inoculated from the knots, and in about two or three days there was abundant growth. From these tubes dilution cultures were made on agar in Petri dishes. Other dilution cultures in Petri dishes were then made from ten of the bouillon tubes, and the uniformity with which one form of colony appeared in the Petri plates seemed to indicate quite conclusively that this was the organism causing the disease. Transfers were then made from these colonies to agar tubes and thence to various culture media.

CULTURAL CHARACTERISTICS.—The organism was grown on the ordinary culture titrated to +1.5 to phenolphthalin and grown at room temperature.

Morphology.—The organism is a motile rod with rounded ends, 1.5–2.5×0.5–0.6 μ. It is usually solitary, but may occur in pairs. Organism direct from the plant as well as from the pure culture shows motility. The size as above given was from a preparation made directly from the tissue.

Agar slant.—On agar growth appeared in about twenty-four hours, as a very thin, grayish-white surface growth. This spread quite rapidly over the surface, especially near the lower portion of the tube. Sometimes small roundish colonies appeared at the side of the growth along the stroke. In cultures a week old the growth is white by transmitted and reflected light. The growth is very thin and scarcely perceptible at first. The condensation water becomes clouded and white.

Agar plate colonies.—On agar plates the colonies become visible after three days. The deeper ones are small, globose, or biconvex with sharp entire margins. The surface colonies are larger and more spreading, circular in outline, whitish in color, and somewhat more dense in center than at margin. The deeper ones often have a straw or cedar color as observed by SAVASTANO. The surface colonies measure 2–4^{mm} in diameter when four days old. They are more vigorous than colonies in gelatin of the same age.

Glycerin agar.—Growth much the same as in agar, except more

vigorous, and hence a thicker surface growth resulted. Numerous small colonies also developed on surface.

Gelatin stab.—Growth takes place along the stab and also on the surface of the media. The growth along the puncture was filiform; on the surface it was thin, spreading from the point of puncture, the margin undulatory to lobate-lobular. No liquefaction.

Gelatin plate colonies.—These appeared in two to three days. The deep colonies much the same as those in agar Petri plates. Surface colonies more spreading, forming a very thin growth with irregular undulatory margins, some darker in color than surface colonies. Surface colonies under low power showed the center to be denser than the margin and finely reticulated. Toward the margin the reticulations are much coarser than in the center.

Potato.—Growth was vigorous and characterized by always being straw color. The growth was quite markedly raised above the surface and soon covered the entire plug.

Bouillon.—In meat bouillon growth appeared after two days as a fine granular substance that remained in suspension. The culture is at first slightly acid to litmus, but becomes alkaline after two weeks.

Glucose bouillon.—Growth the same as in the meat boullion, with the same reactions. The change from acid to alkaline reaction is much slower than in the other media.

Saccharose bouillon.—Growth at first more vigorous than in either the bouillon or glucose bouillon; acid at first, neutral after seven days, and alkaline in two weeks.

Lactose bouillon.—Showed the same general characteristics as saccharose, acid at first, then neutral and alkaline after two weeks.

Litmus milk.—Showed no change until ten days, when there was a distinct alkaline reaction. No coagulation ever occurred. After fifteen days the medium was quite blue, with a slight whitish precipitate at the bottom of the tube. This was not granular, but subgelatinous, and when shaken into solution settled again to the bottom. In a tube two months old the liquid becomes very blue and alkaline.

Milk.—Showed no change except a slight yellowing in color.

Scarcely any experimental work was done in growing the Bacillus at different temperatures. SAVASTANO (**4**) states the optimum temperature for the olive knot to be between 32–38° C, and SMITH (**9**)

has still further restricted the limits of best growth to between 35–37.8° C. Several experiments were conducted by the writer with the oleander organism in temperature about 35.5° C, and no growth took place on agar, bouillon, or potato after four days, although there was good growth in the inoculated bouillon tube at room temperature.

All the liquid media become at first acid to litmus, but change to an alkaline reaction in about two weeks. The media used were all titrated to +1.5 to phenolphthalein. This would be slightly alkaline to litmus, so the growth of the organism caused first an acid, then an alkaline reaction. All the cultural characteristics of the oleander organism, so far as possible, were compared with those described from the olive organism. There seems to be a very close agreement, and without question the organism is identical.

From knots produced by artificial inoculations on olive and oleander the organism was isolated (in the same manner as described before) and grown in the same media as was the original culture from the oleander. These two series agreed perfectly in culture characteristics with one another and with the original culture.

The organism also was isolated from naturally infected olive knots in the same manner as for the oleander. Growth on various culture media showed biochemical and cultural characteristics that agreed with those observed by the writer for the oleander knot; and with those described by SAVASTANO in his study of the olive knot.

This oleander disease is not believed to be a new trouble, but similar to the one found on olives. They are both caused by a motile rod (Bacillus) that grows well on the ordinary culture media, and will cause infection of the olive and the oleander. This infection at length causes characteristic lesions and knot-like growths on the stem and leaves. The knots produced on the olive by the oleander organism agree with typical knots as found on cultivated olives in California, and with various illustrations of the olive knot. The cultural characteristics of the two are similar in all essential respects.

LABORATORY OF PLANT PATHOLOGY,
University of California, Berkeley.

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